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# TEMPERATURE INFLUENCES THE EXPRESSION PROFILING OF IMMUNE RESPONSE GENES IN RAINBOW TROUT FOLLOWING DNA VACCINATION AND VHS VIRUS INFECTION

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A DNA vaccine encoding the glycoprotein (G) genes of the salmonid rhabdovirus viral haemorrhagic septicaemia virus (VHSV) has proven highly efficient against the disease caused by this virus in rainbow trout (*Oncorhynchus mykiss*). Several studies have demonstrated that this vaccine induces both an early unspecific antiviral response as well as a long-lasting specific protection. However, temperature appears to influence immune response with respect to the nature and duration of the protective mechanisms.

In this study, groups of fish were temperature acclimated, vaccinated and challenged at three different temperatures (5, 10 and 15°C). Tissue and organ samples were collected at numerous time points post vaccination (pv) and post viral challenge (pch). Then, gene expression levels of a two immune genes (Vig-1 and Mx3) involved in unspecific antiviral response mechanisms were determined by Q-PCR. The expression profiles appeared similar for the two genes in terms of temperature dependency with a faster induction and shorter duration at the higher temperature. In order to analyze the temperature effect on the relative expression profiles across a larger set of immune genes time points displaying similar Mx3 expression levels post vaccination were identified: 4 days pv (15°C); 7 days pv (10°C); 23 days pv (5°C), respectively.

A targeted trout immune gene array including probes for VHSV genes was used for analysis of vaccinated vs control fish per temperature (4-5 replicates). Temperature altered the transcriptome response to the viral challenge, and the number of responsive genes increased at higher temperature: 51 (5°C), 64 (10°C) and 72 (15°C), respectively, indicating that fish kept at higher temperature may have an enhanced immune response.

Additional correlation analysis allowed identification of genes that were significantly up- or down regulated synchronous with expression of the vaccine G gene. These findings encompass that for example in fish kept at 5°C, putative CD3, CD4, CD9, CD28, CD53, CD63, CD83, CD84 were up regulated, while CD59, CD83 were down regulated, potentially indicating balancing mechanism of the immune system.

An experimental VHSV challenge was performed 7 weeks pv. Similar protection levels of approximately 10% mortality were found for the vaccinated fish, regardless of temperature during immunisation and challenge, whereas the course and level of mortality among the controls were temperature dependent. At day 5 post infection, the number of differentially regulated genes in the livers of vaccinated versus control fish was highest in fish kept at 10°C and lowest in fish at 5°C. This difference presumably reflects the temperature dependent progression of the disease in the controls.

Further analysis of the obtained data with respect to gene regulation pathways as a result of DNA vaccination and/or viral infection will be presented.

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